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Chapter Title: Radioactive Bees–Honey Bees as Indicators of Radionuclide Contamination

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Running Headline: Bees as Indicators of Radionuclide Contamination

Abstract

Many facilities around the world are actively involved in the research and development of nuclear-related materials and the production of nuclear energy. Inherent in the many processes involved in this type of work is the production of radioisotopes. Unfortunately, some of these radionuclide waste products have found their way into surrounding natural areas. The ongoing interest in assessing the influences of contaminants on living systems generates questions on how best to incorporate sampling data into ecological risk assessments. The primary concerns involve determining which methods are best to monitor these contaminants and how to analyze the influences these contaminants have on biological systems. One innovative sampling method incorporates honey bees (*Apis mellifera*) as monitors of environmental contamination. Using honey bees as indicators of radionuclide contamination is an inexpensive form of environmental monitoring, especially considering the numerous sampling points the foraging bees visit. Sampling at one location (the hive) can provide a plethora of information from various points across a landscape relative to the distribution and bioavailability of contaminants. Comparing the concentration of contaminants in the hive products or the honey bees to the known concentrations in the surrounding area can be useful in modeling the redistribution of contaminants through ecosystems. The nature of honey bee ecology makes them an excellent living system from which to monitor the presence of contaminants and explore their impacts.

Keywords: honey bees *(Apis mellifera)*, ecotoxicology, radionuclide, environmental monitoring, contamination

INTRODUCTION

Many facilities around the world are actively involved in the research and development of nuclear-related materials and the production of nuclear energy. Inherent in the many processes involved in this type of work is the production of radioisotopes. Unfortunately, some of these radionuclide waste products have found their way into surrounding natural areas. Historically, sampling for environmental contaminants has been done on the various abiotic components (i.e., water and soil) of an ecosystem and has often excluded the sampling of many of the biotic components. The ongoing interest in assessing the influences of contaminants on living systems has generated questions on how best to incorporate sampling data into ecological risk assessment models. The primary concerns involve determining which methods are best to monitor these contaminants and how to analyze the influences these contaminants have on biological systems. How might we integrate sampling of both biotic and abiotic components of an ecosystem?

One innovative sampling method incorporates insects—honey bees (*Apis mellifera*)—as monitors of environmental contamination. Using honey bees as indicators of radionuclide contamination is an inexpensive form of environmental monitoring, especially considering the numerous sampling points the foraging bees visit. Sampling at one location (the hive) can provide a plethora of information from various points across a landscape relative to the distribution and bioavailability of contaminants. Comparing the concentration of contaminants in the hive products or the honey bees to the known concentrations in the surrounding area can be useful in modeling the redistribution of contaminants through ecosystems. The nature of honey bee ecology makes them an excellent living system from which to monitor the presence of contaminants and explore their impacts.

Past research has demonstrated that honey bees are useful indicators of environmental contamination [10, 6, 23]. Honey bees can be thought of as mobile samplers that efficiently cover a large sample area and then return to a central location [5]. Honey bees forage in an area with a radius as large as 6 km and often cover a total area up to 100 square km [24, 31]. Each hive contains literally thousands of bees, most of whom will forage for nectar, water, pollen, and plant resins, which are all brought back into the hive. During these foraging flights, bees inadvertently contact and accumulate a wide array of pollutants, some of which are brought back to the colony [7]. These contaminants often become incorporated into the bee tissue, the wax, the honey, or the hive itself [32].

Honey bees have been used in the past to monitor the presence and distribution trace elements including fluoride [8,26], lead [27], zinc [9], nickel [1], potassium [2], and the bioavailability of radionuclides [16, 28, 32], including cesium [4, 30], tritium [34, 14], and plutonium [21].

Unfortunately, there are still many gaps in our knowledge concerning the use of honey bees as indicators of radionuclide contamination. More specifically, there are many unanswered questions concerning the dynamics of radionuclide contaminant redistribution through ecological systems. Over the last six years, I conducted a series of field experiments to investigate various aspects of using honey bees as indicators of radionuclide contamination. The goal of this research was to understand the feasibility, including the limitations, of using honey bees as ecological indicators.

In this chapter, I will explore the issue of using honey bees as indicators of radionuclide contamination by reviewing my recent studies conducted at the United States Department of Energy's Los Alamos National Laboratory (LANL). LANL, which is located in north-central New Mexico, has been involved in the research and development of nuclear-related materials for the last five decades.

Field research was conducted at LANL during 1994, 1995, and 1996. The study site is located adjacent to a radioactive waste lagoon that contains known bioavailable contamination including tritium, cobalt-56, cobalt-60, manganese-54, sodium-22, and tungsten-181. I designed a series of field experiments to address a range of hypothesis dealing with honey bees as indicators of radionuclide contamination. These included research into some basic issues, such as comparing the consistency of samples taken from similar bee colonies, to more complex questions such as the dynamics of radionuclide contaminant redistribution through an ecosystem. This chapter is organized into three sections, each summarizing the results of these experiments.

Exploring Colony Variability and Temporal Contaminant Accumulation

Several experiments were designed to investigate in greater detail the use of honey bees as monitors and explore various aspects related to the sampling these bees. Colonies of bees have been shown to contain radionuclide contaminants when the contaminants are environmentally available. However, there are many unanswered questions, notably those dealing with sampling protocol issues and the interpretation of data. The purpose of the study described in this section, was to explore two issues concerning sampling protocol and the interpretation of data collected from colonies of differing ages. Two separate experiments were conducted; the first tested inter- and intracolony variability. Do bee tissue samples taken from similar colonies under similar conditions yield the same results? The second experiment tested the hypothesis that there is no difference in contaminant levels found in colonies of varying ages located in the same area. In other words, is there a temporal accumulation of contaminants within colonies that needs to be considered when interpreting the sample results? Results from these experiments are reported in Haarmann 1997 [18].

Two separate field experiments were conducted at a LANL study site with bioavailable radionuclide contamination. A series of honey bee samples were collected from colonies, analyzed for concentrations of radionuclides (gamma-emitting nuclides, uranium, and tritium) and the results were then compared using graphical and statistical methods. The first experiment, which examined variability of sample results, indicated that generally a low variability in radionuclide concentrations existed between samples collected within the same colony. Furthermore, results indicated that a higher variability existed between samples that were collected from adjacent colonies within the same study site.

Some interesting aspects relative to radionuclide concentrations were discovered during these experiments. For example, the concentrations of tritium and sodium-22 found in samples taken from similar colonies were inconsistent, while levels of cobalt-57, cobalt-60, and manganese-54 were consistent. I will discuss the significance of these finding later in this section.

A second experiment investigated the accumulation of radionuclides over time. In this field study, I collected samples from colonies that had been located in a contaminated area for several years and from colonies that had been placed in the study site several months earlier. This experiment demonstrated that there was indeed a significant accumulation of radionuclides within colonies over time. Below is a discussion of some conclusions that can be drawn from the findings of these experiments?

Colony Variability Study

The results of this experiment confirmed the findings of many other studies demonstrating that honey bees are good indicators that contamination is bioavailable [11, 5, 14]. Generally, samples taken from the same colony and the same area of the hive, i.e. honey frames, displayed small variability. This may suggest that collection of a single sample from a colony adequately represents the colony for that point in time and the bees in that area of the hive. However, if honey bees are to be considered a serious method of radionuclide contaminant monitoring, there needs to be evidence of low variability among adjacent colonies when the exposure source is consistent. The results demonstrated an interesting trend in intercolony variability: all samples taken from colonies of the same age, showed no significant differences among radionuclides, except tritium and sodium-22.

The tritium and sodium-22 samples were all significantly different from each other. There are numerous explanations for this, a few of which merit further experimentation. One explanation relates to the dynamics of tritium and sodium-22 in the bee's body. Since both hydrogen and sodium are involved in several physiological processes and are readily transported through the bee's body, the total contaminant amount per individual is likely influenced by such factors as temperature regulation, spatial and temporal foraging patterns, energy expenditure, and flight activity. In areas where the rate of exposure to colonies is consistent, there may be greater differences in the concentrations of those elements that are active in physiological processes than in concentrations of elements that are less active. My preliminary research has indicated that this was the case.

The samples collected from the older colonies were significantly different from each other. This is not surprising considering that the older colonies group actually consisted of two colonies of different ages. The older of the two old colonies had consistently higher and significantly different levels of contaminants. This fact lead me to further investigate the possibility of a temporal accumulation of contaminants.

Temporal Contaminant Accumulation Study

There was a significant difference between radionuclide samples taken from different aged colonies. Colonies that had been in the exposure site more years, had consistently higher levels of radionuclide contamination than newer colonies. Research has shown that radionuclide contamination is found in bee tissue, honey, pollen, and wax [22, 32]. In colonies that are located in a contaminated area for several years, contaminants are likely passed to young bees via trophallaxis and direct contact, prior to any foraging activities. Thus, when an individual bee begins to forage, she is already contaminated with radionuclides. Likewise, during the winter months, bees in these colonies are feeding on contaminated honey. This "pre-contamination" of foragers results in tissue samples that show higher levels of radioisotopes than are actually available to the bees during foraging.

In the case of those radionuclides with a half-life that exceeds one year, the contaminants potentially remain in the colony for several years. Thus, the longer a colony remains in a contaminated area, the greater the accumulation of radionuclide contaminants. Subsequently, bee tissue samples from older colonies would be expected to have higher levels of radionuclides.

Futhermore, one of the new colonies had higher levels of tritium than one of the old colonies. Likely, there are many variables that can influence the levels of contaminants in a colony, bioavailability being only one of them. The fact that a new colony would have higher levels than an old colony suggests that there is a complicated interplay between these variables. For example, the degree in which a contaminant is involved in metabolic processes may be more important in determining the levels in the colony than does the age of the colony. In the future, it will be important to further investigate these variables, determine how they influence one another, explore how they effect the amount of contamination in a colony, and ultimately take this information into account when interpreting the data.

Comparative Studies of Contaminant Levels in Forager and Nurse Bees and in the Flowers of Three Plant Species

I conducted two experiments to examine the contaminant levels in forager and nurse bees and the contaminant levels in the flowers of three different plants. These experiments were designed to investigate two factors that might influence the levels of contaminants found in a standard sample of honey bees (generally 1,000 bees) used for monitoring purposes. The first experiment tested the contaminant level differences in forager bees versus nurse bees. Might the proportion of forager bees to nurse bees in a particular sample influence the contaminant levels found in that sample? While the second experiment compared the levels of contaminants in three floral species used frequently by foragers. Might differences in the proportions of these species used as forage influence the levels of contaminants found in the bees? Results from these experiments are reported in Haarmann 1998 [19].

As previously mentioned, in addition to being indicators of bioavailable contaminants, honey bees potentially are a good model from which to explore the redistribution of contaminants from abiotic components such as water and soil into biological systems. However, before honey bees can be fully used in this context, more information is needed concerning the dynamics of radionuclide contaminant uptake in honey bees and the factors that ultimately determine levels of radionuclide contamination in the bees [17].

The experiments were conducted within a study site containing radionuclide contamination above background levels. The first experiment compared levels of radionuclides found in forager bees to those levels found in nurse bees. Bees were collected from colonies, analyzed for concentrations of radionuclides (gamma-emitting nuclides and tritium), and the results were compared using graphical and statistical methods. Results indicated that there were no significant differences between the contaminant levels in forager and nurse bees.

A second experiment compared the levels of radionuclides (gamma-emitting nuclides and tritium) found in the flowers of three plant species growing in the study site: salt cedar (*Tamarix ramosissima*), white sweet clover (*Melilotus albus*), and rabbit brush (*Chrysothamnus nauseosus*). Results from this experiment indicated that there were no significant differences in the amounts of radionuclides found in the flowers of these three plants. A discussion of the significance of these studies is presented below.

Forager/Nurse Variability Study

The results of my experiment indicated that there were no significant differences between the levels of radionuclide contaminants found in forager bees and nurse bees. It is possible that equilibrium is reached between the levels of contaminants in foragers and nurse bees. In experiments with radioactive nectar, Free [13] demonstrated that over 75% of foragers involved in food exchange contained the radioactive nectar after 24 hr. Using colored and radioactively labeled nectar, Nixon and Ribbands [29] showed that over 50% of a colony's workers contained the tracer nectar only 24 hr after 10 foragers had brought it into the colony. Assuming that contamination is spread through the colony very quickly, equilibrium between the levels of contamination in the foragers and nurse bees should be achieved within a short period of time.

Nurse bees most likely acquire the majority of their contamination from water and nectar they receive from foragers during trophallaxis. In addition, since larvae are partially being fed a contaminated food source, emerging adult bees are likely already contaminated before they receive the incoming contaminated nectar or water.

Worth noting is the fact that there was an interesting graphical trend relative to the levels of several radionuclides observed in the bees. Nurse bees tended to have slightly higher levels of beryllium-7, sodium-22, tungsten-181, and tritium. However, one might expect the foragers, not the nurse bees, to have higher levels of contamination because they (1) are older than the nurse bees and have had the longest time exposure to the contamination and (2) continually come in direct contact with the contamination sources while foraging.

Radionuclides tend to follow pathways similar to the nutrient analog [33]. The graphical trend of nurse bees with slightly higher levels of contaminants seen in this experiment supports study discussed previously suggesting that radioisotopes of physiologically important elements such as hydrogen and sodium are readily transported through the honey bee's body [18]. The levels of these contaminants in a bee's body may be influenced by such factors as flight activity, temperature regulation, spatial and temporal foraging patterns, and energy expenditure. Forager bees possibly expel sodium-22 or tritium via respiration during situations like foraging, that require increased metabolic activity. The increased metabolic activities of forager bees may ultimately contribute to slightly lower levels of certain contaminants in forager bees than in nurse bees.

Another interesting discovery was the fact that the nurse bee sample results showed a greater standard deviation, compared to the forager samples. This was likely a result of the range in age structure within each nurse bee sample. Although highly variable, the pre-foraging activities of nurse bees generally occur between the ages of 1-3 weeks, while foraging activities often begin around 3 weeks of age. However, the average worker forages for only 4 or 5 days before she dies [35]. My previous study comparing bees from colonies located near the radioactive lagoon demonstrated that bees sampled from colonies located in the area several years had significantly higher levels of radionuclides than bees in colonies located in the area for only several months [18]. The level of contaminants in a honey bee is likely directly related to the duration of the exposure.

Amongst the nurse bees of a colony, the younger bees are less contaminated than their older sisters. One would assume that the nurse bee samples should have a greater standard deviation than the forager samples because the nurse bee samples contain bees with a larger age range; this is indeed the case. The levels of contaminant in a single nurse bee sample would reflect the proportion of younger nurse bees to older nurse bees.

Floral Variability Study

Theoretically, it is possible that a variation in floral contaminant levels might affect the levels contained in honey bees that forage on those flowers. However, my experiment demonstrated that there were no significant differences in the levels of contaminants in the flowers of the three main forage plants. Probably, the species of flower the bees had visited had little influence on the levels found in the bees. In addition, the uptake of contaminants via flowers may have contributed little to the overall levels in the honey bees, since there was a radioactive waste lagoon nearby that contained much higher levels of radionuclides. Because bees collected water from the lagoon, they conceivably accumulated most of their contaminants from the water rather than from the nectar of surrounding flowers. Although the particular species of flowering plants used as forage in my study did not appear to have significantly influenced the radionuclide concentrations found in the bees, there were some notable graphical trends.

Although salt cedar is halophytic (e.g., grows in saline soil), the concentration of sodium-22 in the salt cedar flowers was very low. Like it's nutrient analog, sodium-22 is probably readily absorbed by salt cedar and accumulated in the leaves. We know that salt cedar increases surface soil salinity by transporting salts to the leaves and subsequently releasing these salts back into the surrounding soils when the leaves are shed [3], thus giving it a competitive advantage over non-halophytic plants [12]. Thus, it is likely that the majority of sodium-22 is being partitioned into the leaves rather than the flowers.

The lowest mean levels for three of the six contaminants (sodium-22, cobalt-57, and tritium) were observed in salt cedar, while the lowest mean levels for the other three contaminants (manganese-54, tungsten-181, and beryllium-7) were observed in the sweet clover. Rabbit brush, on the other hand, had the highest levels for three of the six contaminants (manganese-54, beryllium-7, and tritium). This is consistent with studies conducted by Fresquez *et al.* [15], which demonstrated that rabbit brush tends to readily take up radionuclides (Sr-90 and U) in contaminated sites. While salt cedar and rabbit brush are perennials and sweet clover is an annual, there did not appear to be a clear correlation between the accumulation of contaminants in these plants and their life cycle.

In conclusion, these experiments contribute to an understanding of the factors that may ultimately influence the concentrations of radionuclide contaminants in honey bees. The experiments demonstrated that within the study site, the particular temporal caste of honey bee collected for analysis does not significantly effect the results. In addition, the various species of flowers within the study site do not contain significantly different concentrations of radionuclides. Therefore, the particular species of flower used as forage does not significantly influence the overall concentrations of radionuclides in the bees.

Investigating Contaminant Redistribution Using Concentrations in Water, Flowers and Honey Bees

This section describes a two-year study that examined the movement of contaminants within an ecosystem. The purpose of this study was to investigate the redistribution of contaminants within a study site as the contaminants move from the source, in this case a radioactive waste lagoon, to the honey bees. My experiments were designed to investigate several questions: (1) Do the bees take up the majority of contaminants from the lagoon or from nearby flowers? (2) Are the levels of contaminants in the bees, flowers, and water correlated, and do they demonstrate similar trends? and (3) Is there an observable bioaccumulation of contaminants within the bees or flowers? Results from these experiments are reported in Haarmann 1998b.

As I mentioned earlier, besides being indicators of bioavailable contaminants, honey bees may prove to be a good model from which to explore the redistribution of contaminants within ecosytems. But, more information is needed concerning the dynamics of radionuclide contaminant uptake in honey bees and the factors that ultimately determine levels of radionuclide contamination in the bees, including biomagnification and bioaccumulation.

In this series of experiment, I took samples of water, flowers, and honey bees from the contaminated study site for two consecutive years. The samples were analyzed for concentrations of radionuclides (tritium and gamma-emitting nuclides), and the results were compared using rank sum, correlation, and trend analysis. Results were then used to assess the redistribution pathway of radionuclides within the study site. Results indicated that honey bees received the majority of their contamination directly from the source—a radioactive waste lagoon. The amount of contamination the honey bees received from flowers during nectar collection appeared to be insignificant compared to the amount received during water collection. Results did not demonstrate significant patterns of correlations or trends between the lagoon, bees, or flowers. Sample results showed a significant bioaccumulation of cobalt-60 and sodium-22 within the honey bees, but no significant bioaccumulation within the flowers. Several important issues were discovered as a result of these experiments.

Previous studies at LANL have investigated the redistribution of radionuclide contaminants within the environment. Hakonson and Bostick [21] measured the contaminant levels of tritium, cesium-137, and plutonium in bees, honey, surface water, and vegetation. The authors concluded that tritium levels in bees appear to equilibrate with the source. Cesium-137 and plutonium concentrations were low or undetectable in the bees during this study, and therefore, difficult to use in the analysis. The authors suggested that because there appeared to be several locations from which the bees received the radionuclides, it was difficult to interpret the data and understand patterns of redistribution.

In my experiments, because the lagoon was the only major source of tritium, it is easier to understand the redistribution of tritium within the study site. Because the levels detected in the flowers were consistently less than those present in the bees, and because the lagoon levels were consistently higher than the levels in the bees, the bees were receiving the majority of their tritium from the lagoon, with much less being contributed by the flowers. In areas with lower source levels, the redistribution patterns would certainly be different, including the possibility that the flowers would be a significant contributor of tritium to the bees.

Consistently, the floral samples contained the lowest levels of all contaminants. The levels were all significantly lower than those observed in either the lagoon or the bees. These results are to be expected since the majority of plants in the study site were not taking up the contaminants directly from the lagoon water; therefore, the redistribution of contaminants to the plants in the area was somewhat limited.

The levels of cobalt-60 and sodium-22 detected in the bee samples were significantly higher than the levels in the lagoon samples. As part of an ongoing LANL surveillance program air, water, soil, and foodstuffs were monitored in the study site [25]. These studies indicated that the only major source of cobalt-56, cobalt-60, manganese-54, sodium-22, and tungsten-181 near the study site was the waste lagoon. Because the bees were only receiving cobalt-60 and sodium-22 from the lagoon, and because the levels found in the bees were significantly higher than those at the source, it is apparent that bioaccumulation of sodium-22 and cobalt-60 was occurring within the honey bees. There was not a significant bioaccumulation of radionuclides within the floral samples.

The correlation analysis did not detect significance, I would not rule out the fact that a relationship may exist between the levels of contaminants in the lagoon and those in the flowers and bees. Analyses indicating "no significant correlations" in the contaminant levels, may simply be a result of my small sample size and the difficulties associated with detecting correlations of data sets with small sample sizes. The strongest positive correlation appears to be between the levels of contaminants in the lagoon and the bees. This is in agreement with the findings of my statistical analysis that indicated the lagoon is the primary source of contamination for the bees. Similarly, Fresquez et al. [14] examined 17 years of data on the tritium levels in honey and bees. This study found no significant correlation between the levels in the bees and the honey.

A trend analysis indicated that, for the most part, upward trends were seen in the lagoon and the bees for all the contaminants. This further supports the hypothesis that the bees were likely receiving the majority of their contamination from the lagoon. The floral samples showed a variety of trends. For example, the first-year tritium lagoon and flower trends showed upward trends, while the next year they showed opposite trends. In fact, for most cases the flowers and lagoon showed opposite trends.

In conclusion, while trend and correlation analysis did not result in statistically significant findings, the bioaccumulation of certain radionuclides within the honey bees was apparent. As for understanding the dynamics of contaminant redistribution, perhaps larger sample sizes will be needed in future studies so that correlation and trend analysis may prove more statistically powerful. Additionally, it would be useful in future studies to analyze nectar itself rather than the whole flower. Samples could also be collected from air and soil within the study plot to further assess other potential pathways. Nonetheless, this study is helpful in understanding which point sources significantly contribute to the levels of contamination within the bees, as well as the issue of bioaccumulation of certain radionuclides within the honey bees.

SUMMARY

As discussed in this chapter, the finding of these experiments verify that honey bees are indeed good indicators of radionuclide contamination when it is present in the environment. In addition, the data provide insight into those factors that contribute to the overall levels of contaminants detected in the honey bees. These factors include such issues as temporal contaminant accumulation, the type of plant species used as forage, and the redistribution of contaminants within ecosystems.

On a more applied level, my research contributes a better understanding of the use of honey bees as indicators of contaminant availability. The findings of these experiments can assist in: The planning and study design of projects that will use honey bees, the management of honey bee colonies when used in monitoring projects, and the establishment of protocols for sample collection.

Currently, the real challenge that faces us, lies in incorporating this type of sampling data, into ecological risk assessment models. How good are the data? Can we meaningfully interpret the analytical results? Are honey bees a good species to use? These are but a few of the issues we will grabble with if we want to successfully use honey bees as indicators of environmental contamination.

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